DISTRIBUTION OF TRICHINELLA SPIRALIS IN THE RECTUS EYE MUSCLES OF THE LABORATORY WHITE MOUSE

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INTRODUCTION AND REVIEW OF THE LITERATURE

Man, because of his increasing population, has found it necessary to seek a more efficient means of producing a sufficient food supply. The ability of parasites to take advantage of every possible road to a successful completion of its life cycle, has made stringent controls in the production and distribution of foods important. Trichinella spiralis has been able to complete its life cycle in North America in various ways, in spite of the high standards of sanitation. Blair, et al. (1969), studied the incidence of trichiniasis in Cincinnati, Ohio, and found that 4.86 per cent of the 332 human autopsies studied showed the presence of trichiniasis. Zimmerman (1968), made a nation-wide study of the incidence of trichiniasis. Of 4,562 human autopsies in 33 states, 4.1 per cent were found to have trichiniasis. Kagan (1962), in his review paper, makes a plea for continued research for a means of controlling trichiniasis.

The first documented observation of trichiniasis, according to Reinhard (1958), was in 1835, by a graduate student, James Paget. Paget observed the larvae of Trichinella spiralis in human muscle during an autopsy. During the same year, Owen named the parasite Trichina spiralis. Because the name Trichina had been used to describe another animal genus, the name was changed to Trichinella spiralis (Gould, 1945).

Verchow determined the life cycle of Trichinella spiralis in 1859 (Reinhart, 1958). Trichinella spiralis is an obligatory nematode parasite which spends its entire life in the same host. The female Trichina worm is ovoviviparous and the progeny are developed in-utero (Gould, 1945). The female is larger than the male, and the male is usually shorter lived than the female (Chandler, et al., 1961).

The life cycle has been summarized in review papers by several authors (Gould, 1945; Kagan, 1962; Larsh, 1963). After infected meat is eaten, the encysted larvae escape from their encapsulation into the small intestine of the new host. Generally within two days the worms reach sexual maturity (Gould, 1945). The worms receive their nutrition from the tissue juices in the small intestine. The developing adult Trichina worms reside in the mucosa of the villilary folds (Gould, 1945). Larsh (1963), reports that in mice the pre-adult period lasts 33 hours. The gravid female mice give birth to the young larvae deep in the mucosa so that they are not lost in the lumen material (Gould, 1945). The duration of time between infection and birth varies in different hosts but is between four and seven days. In smaller mammals, Trichina seem to have a faster rate of development (Gould, 1945).

The young minute motile larvae burrow through the intestinal tissues and enter into the circulatory system. The part of the circulatory system which is invaded first

is questionable. Most authors agree that the larvae eventually enter into the peripheral blood supply. From this point the larvae are distributed to all parts of the body. Burrowing through the tissues of the circulatory system, the larvae enter into the musculature. At this point, the larvae mature and become encapsulated (Gould, 1945).

Larsh (1963), indicates three phases of the life cycle: the gastro-intestinal, the circulatory, and the muscular phases. At four days postinfection, there is a mild inflammatory response in the anterior portion of the small intestine. This inflammation increases in intensity and eventually subsides. The age of the mice is important symptomatically, resulting in varying degrees of fever, diarrhea, anorexia, weight loss, and a general reduction of activity. The gastrointestinal response lasts approximately 14 days reaching its peak from six to nine days (Larsh, 1963).

The presence of the larvae and the adults results in an antigen-antibody cardiovascular response. In infected mice, there is an inversion of the lymphocyte and neutrophil ratio (Larsh, 1963). The neutrophils gain in numbers and eventually become more numerous than the lymphocytes. At 19 days postinfection, there is a low grade eosinophila in mice (Larsh, 1963). Leonard, et al. (1941), report that Trichina larvae are first found in the circulatory system during the seventh day postinfection. In man, the larvae are most

numerous in the blood stream between 8 to 25 days postinfection (Chandler, et al. 1961). Larsh (1963), found that after 7 to 11 days, few larvae are found in the blood stream of mice. He concludes that in mice the larvae invade the musculature very rapidly. The number of larvae which enter into the circulatory system in mice may depend on the rate of "emptying the bowel" (Larsh, 1963). It appears that this could explain the slight immune response in younger mice.

The final phase of development is the muscle phase. Matoff et al. (1962), recognized three stages of larval development: the young larvae in the circulatory system, the larvae invading the striated muscle, and the mature encapsulated larvae. In mice at about the 14th day, myositis develops in the muscle tissue (Larsh, 1963). It increases in intensity and reaches a peak at 24 days postinfection (Kozar, 1963). By the 30th day postinfection the myositis has subsided (Larsh, 1963). The developing larvae may cause a chemical breakdown and depletion of host muscle proteins which is probably due to the production of aminopeptidase (Larsh, 1963).

The review of clinical symptoms is important, because it may suggest the sites of invasion. In man, the early symptoms of the second stage of trichiniasis include swelling of the eyes and the lids and invasion of the skeletal muscle causing intense muscular pain (Chandler et al. 1961). Pain associated with eye movements, during this stage of

the disease, may indicate that these muscles have been invaded (Chandler et al. 1961). Lehrfeld (1940), and Croll (1952), report symptoms which indicate the involvement of Trichinella in the eye muscles of humans. A swelling of the eyelids and chemosis of the bulbar conjunctiva are the most common symptoms. Optic neuritis, edema of the retina, and inflammation of the external eye muscles were also mentioned (Croll, 1952). Salan et al. (1928), and Croll et al. (1952), make special mention of the involvement of Trichinella in the medial and lateral rectus eye muscles.

The anatomy of all vertebrate eyes is very similar (Weickert, 1967). Harderian glands are unusual in most mammals but are found in mice (Weickert, 1967). The embryonic development of the eye muscleshas been established and the six extra-ocular eye muscles are the same throughout the vertebrata (Atwood, 1962).

The six extra-ocular eye muscles are found in two groups which include the four rectus muscles and the two obliques. Together their function is the coordinated movements of the eyes (Alpern, 1962). The muscles of concern in this present study are the four rectus muscles. All four of these muscles have a common origin, the common tendonous membrane called the Annulus of Zinn which is located at the apex of the orbit of the skull. The muscles diverge as they pass the globe of the eye and insert into the sclera of the eye (Alpern, 1962).

The medial rectus is located toward the inner surface of the skull on both sides of the nose. The lateral rectus is located on a horizontal plane opposite the medial rectus. The superior rectus is located on the superior surface of the globe. The inferior rectus is found at vertical angles to the superior rectus (Alpern 1962).

The distribution of the larvae is important to this study. The larvae migrate through the circulatory system at which point they burrow through the tissues as mentioned previously. The question arises as to the choice of a place of residence for the larvae. Britov (1966); Chandler et al. (1961); Gould (1945); Hill (1957); Larsh (1963); and Matoff et al. (1962), all agree that Trichinella spiralis larvae show a prediliction for striated muscle. Experiments by Hill (1957); Kershaw et al. (1956); Leonard et al. (1941); and Lukashenko (1962), all verify that the larvae of Trichinella spiralis are found in organs other than the striated muscle. Even though the Trichina larvae have been found in organs other than the striated muscles, when tested by Britov (1966), it was found that the encapsulated larvae only show normal development. Since the larvae do not encapsulate in these organs devoid of striated muscle, the Trichinae of striated muscle are the only larvae important in the epidemiology of the disease (Olson, 1964). The larvae found in organs devoid of striated muscle are noninfectious (Matoff et al. 1962). The reason for the location of some larvae

may be explained by the massive doses given to the experimental organism (Zimmerman et al. 1961).

The question as to which striated muscle or muscle group is the most highly parasitized has been studied by several authors. In swine, the crura of the diaphragm is most highly invaded by Trichina larvae (Hill, 1957; Matoff et al. 1962; Zimmerman et al. 1961). Studies involving mammals other than swine indicate some variation. In the cat, the lingual muscles have the greatest density per gram of muscle tissue (Groeglyad, 1963); whereas in the monkey, the biceps has the greatest density per gram weight (Nelson et al. 1962). Oliver (1962), found that in rats the diaphragm was most highly parasitized followed by the extrinsic eye muscles. Schoop et al. (1961), completed a study involving rats, rabbits, and mice. In this study, it was found that in rats and mice, the eye musculature contained more than two times the Trichinae per gram of muscle tissue. The results of this study agree with Lukashenko's (1962), observation that the eye musculature is highly parasitized. In their study, Schoop et al. (1961), found the ratio of larvae in eye musculature to gastrocnemius per gram to be 2.07:1.

The present study was concerned with the effect of dosage and the concentration of Trichinella spiralis larvae in the rectus muscles of the eye. Olson (1964), reviews the work done on the concentration of larvae recovered from

swine after experimental infection. The range of larvae recovered is 36 to 200 times the number fed (Chandler et al. 1961). Chandler et al. (1961), points out that studies vary but indicates that each gravid female produces about 1500 larvae which will become encysted.

Another problem still unresolved is the correlation between dosage and number of encysted larvae and its effect upon distribution. Most authors agree that there is a correlation between number of larvae ingested and the number found in striated muscle (Matoff et al. 1962; Zainman, 1960; Zimmerman et al. 1961). Dykova (1964), disagrees with these findings. Olson (1964), points out that the correlation exists but with a wide variation, dependent on the length of time the adult worms are in the intestine, the immune response, and the severity of the infection. It was also noted that each individual organism had individual differences which affect susceptibility (Nelson et al. 1964).

The knowledge of larval densities is important to man for the determination of the best site for biopsy and for postmortum examination (Nelson et al. 1964). This knowledge is important for the determination of which organ to use for inspection of meat products as a public health service (Matoff et al. 1962). There are two standard methods for determining postmortum incidence of trichinaisis, the use of artificial digestive process and the trichinoscopic method. Using the artificial digest method, the entire

muscle can be used and the total number of larvae assessed. In the second method, one gram samples of the muscles are used and studied microscopically. The degree of infection can then be determined by knowing the total weight of the muscle. Today the trichinoscopic method is used where pork products are inspected (Matoff et al. 1962). For experimental use, Blair et al. (1969), indicates that both methods give similar results. Olson et al. (1964), mentions that there was little difference between the two methods.

Another consideration should be in the study of the distribution of larvae within the muscle itself. Olson et al. (1964), indicates that the site of encapsulation is not random or evenly scattered, but rather localized near the tendonous fibrous portion of the muscle. Carter (1930), reported that Herrenschwand observed the greatest density of larvae in the belly of eye muscles.

The purpose of this investigation was to determine the distribution of Trichinella spiralis in the rectus eye muscles of experimentally infected mice. There have been many studies on the distribution of Trichinella, but only single observations as to the distribution in the eye musculature (Schoop et al. 1961). Most of the previous data concerning infectivity of eye musculature is intuitive using symptomatic clinical data.

METHODS AND MATERIALS

The strain of white mice used in this investigation was maintained at Drake University. The mice were of the same approximate age and weight. The mice were maintained on Purina Laboratory Chow and water ad libitum. They were housed in plastic vivaria using white pine saw dust as a base. The mice were divided into three groups, five mice per group and housed by group, five per vivarium.

The strain of Trichinella spiralis used in the investigation was a strain maintained at Drake University. The trichinella larvae were harvested using the following method. The mice were sacrificed by cervical disjuncture. A 2 cm. incision was made in the mid-ventral abdominal surface and the skin removed. The feet, tail, and head were excised and discarded. Another ventral abdominal incision was made laterally to open the abdominal cavity. The mice were then eviscerated and samples of muscle tissue examined to determine the degree of infection. The lungs and heart were removed from the thoracic cavity leaving only the skeleton and the attached musculature. The body was then cut into several pieces.

A tapwater solution of 1% HCL (Fisher reagent ACS) and a 0.5% pepsin (Fisher NF) was prepared. The chopped mouse and 100 ml. of digest solution was blended (Waring) for 30 seconds at 17,000 r.p.m. (Gallogly, 1968). This

solution was then poured into a 250 ml. screw top erlenmeyer flask and another 150 ml. of the digest solution was added. The digest was maintained in a water bath (Thelco model 85) at 37°C. for eight hours. The solution was mixed occasionally during this time. At the end of this period, the digest solution was poured through two layers of cheese cloth (Curity grade 50) into another erlenmeyer flask. The contents were allowed to settle for 15 minutes. All but approximately 50 ml. of the fluid was then removed using an aspirator. Tapwater at 37°C. was added to return the volume of the liquid in the flask to approximately 250 ml. This was allowed to settle for 15 more minutes and the procedure was repeated four times. Fifteen minutes after the last repeat of this procedure, the excess fluid was removed leaving approximately 50 ml. in the flask. The remaining solution was poured in equal volumes into 15 ml. centrifuge tubes and centrifuged (Fisher Scientific Safety) for three minutes.

Each centrifuge tube was then placed in the 37°C. water bath. The centrifuge tubes were used one at a time until the source of larvae was depleted. Immediately before being used, the excess fluid in each tube was removed by aspiration.

Using a medicine dropper pipette, a drop of the concentrate was added to two depressions on a spot plate. A dissecting microscope with a zoom lens (Bausch and Lomb,

10-25X) was used to count the number of larvae in each depression. When the larvae were counted, the drop was then pipetted into a depression slide. The spot plates were then observed using the dissecting microscope to determine the number of larvae which remained on the plate. This number of larvae was noted and subtracted from the total.

When the proper number of larvae were added to the depression slide, the pipette was rinsed in approximately 2 ml. of tapwater. This rinse was then examined by using the dissecting microscope. Again the number which remained in the rinse was subtracted from the total. The total was maintained at no more than ten over the required number of larvae.

The mice were lightly anesthetized with ether (Merck USP). An elongated pipette charged with the concentrated larvae fluid was forced down the esophagus by pressing lightly and turning at the same time. The contents of the pipette were then forced into the stomach. The pipette was then flushed with 2 ml. of water and this was checked microscopically for the larvae. This amount was subtracted from the total. The total was recorded. The mice were observed carefully to determine if the fluid was retained. None of the mice in this investigation showed adverse effects from this treatment.

The mice were divided into three groups. During the preliminary investigation three mice were examined which

had been infected with tapwater. This group served as the control group. Group one was infected with 100 larvae each. Group two was infected with 200 larvae each. Group three was infected with 300 larvae each.

The mice were maintained for 30 to 36 days postinfection. Following this period of time, the mice were sacrificed. The mice were dissected individually and the data tabulated before another mouse was sacrificed to insure that the specimens were fresh.

Each mouse was sacrificed by terminal exposure to ether and then weighed to determine the total weight. A 2 cm. incision into the skin was made in the mid-ventral abdominal surface. The skin was removed from the abdomen, thorax, and head. The head was removed at the base of the skull. The head was placed into a 50 ml. flask and covered with foil. A transverse incision was made into the abdomen being careful not to cut into the rib cage. The viscera attached to the abdominal side of the diaphragm was removed. A small incision was made into the left side of the rib cage providing a small hole in the diaphragm. Using forceps and iridectomy scissors, it was possible to carefully cut the diaphragm from the abdominal wall. The viscera on the thoracic side was cut away and the diaphragm removed. The diaphragm was observed using the dissecting microscope and using watchmakers forceps the extraneous viscera was removed. The diaphragm was weighed (Mettler Balance). Using standard

microscope slides, the diaphragm was spread out and cut in half. Each half was pressed between two slides. The muscle presses were then observed microscopically (Graph-Apsco Microscope, 3.5X objective, 10X ocular). The number of larvae were counted using a mechanical stage and a key press mechanical counter (Clay-Adams). Each half of the diaphragm was counted once and the total number of larvae in the diaphragm was recorded.

The head was cut in half longitudinally using a scalpel. Each half of the lower jaw and brain was removed. The left side of the skull was always dissected first. The skull was pinned to a small piece of parafin using number 1 insect pins.

Using the dissecting microscope, watchmakers forceps (Clay-Adams) and iridectomy scissors, the skin and fascia were removed from around the eye. Very carefully the conjunctiva connecting the globe to the orbit was removed. The eye was then observed to determine the condition of the extraocular eye muscles. Next the zygomatic arch and maxilla were removed using forceps and scissors. At this point the insertion of the inferior rectus is visible. Using iridectomy scissors, the muscle ligament was cut to make sure the muscles would be identified correctly. The globe was carefully pulled away from the parietal and frontal portion of the orbital plate. Using the point of a scalpel, the portion of the orbital plate around the origin of the rectus

muscles (i.e. the Annulus of Zinn) was cut away leaving the globe and the muscles free.

The eye was pinned to a fresh piece of parafin making certain that the inferior rectus was in the same relative position as it was when it was in the skull. Methodically each of the four rectus muscles were removed, first by cutting the ligement at the insertion (the globe) and next by teasing the muscles apart with watchmakers forceps at the origin. Each muscle was placed into a drop of tapwater on a standard microscope slide which was previously labeled. A cover slip was placed on top of the muscle and using a rubber bulb of a medicine dropper, the cover slip was pressed downward thus pressing out the muscle tissue.

Each muscle was observed microscopically under 35X magnification and then carefully with 100X magnification. The number of Trichinella spiralis larvae in each muscle was determined. The site of the larvae (i.e. near the ligament or the belly of the muscle) and whether they were encapsulated or non-encapsulated was recorded. The cover slips were then removed, and the muscles were placed on a dry slide. The excess water was blotted, and the muscles were weighed collectively.

DATA AND DISCUSSION

The mice used in this investigation were observed each day postinfection. During the first week there was a noticeable change in the fecal wastes in the experimental mice. The regularly hard excrements were soft indicating diarrhea. This persisted for only a short time. From the tenth day until the mice were sacrificed, the mice appeared to be normal. There was a weight gain during this period which also indicates normal development. There was no loss of hair, a symptom sometimes accompanying a harsh dose of Trichinella. None of the mice suffered observable changes in development due to the experimental procedures.

As a corollary with the study of the actual distribution and frequency of Trichinella spiralis larvae in the eye muscles, the diaphragm was used as a guide to the degree of infection. The data in Table 1 compares the larval frequency in the total rectus eye musculature and the diaphragm. The data includes the weight of the mouse immediately before dissection. There is no positive correlation between the weight of a mouse and the number of larvae found in the eye musculature or diaphragm.

In the discussion which follows, those mice which were infected with 100 larvae will be referred to as group A, 200 larvae group B, and 300 larvae group C. Table 1 does not show the number of larvae per individual eye muscle.

TABLE 1. Frequency of muscle trichinella larvae in the diaphragm and eye musculature

Mouse	Wt. g.	No. of larvae introduced	Dphm. wt. g.	Dphm. count	Eye muscle count	Eye muscle wt. mg.
1	34.1	100	.2324	486	3	9.2
2	33.9	100	.1567	264	1	12.6
3	35.2	100	.1587	538	5	13.7
4	29.6	100	.1417	500	4	13.7
5	31.8	100	.1789	374	2	15.1
6	30.9	200	.1902	1056	15	7.7
7	31.6	200	.1668	1040	5	9.5
8	28.9	200	.1619	937	3	9.6
9	29.8	200	.1414	1028	8	10.6
10	26.6	200	.1218	834	15	10.9
11	21.7	300	.1331	1093	3	12.5
12	29.0	300	.2111	856	18	11.7
13	35.9	300	.2232	1380	17	12.0
14	37.9	300	.1785	1013	9	9.8
15	32.5	300	.1900	1414	13	10.8

There appears to be no relationship between the size of the mouse, and either the weight of the diaphragm, or the weight of the eye musculature.

Table 2 was prepared from the data of Table 1. It shows the significance of the range of differences in the degree of infection for the three groups of mice. The range of difference in case one was highly significant and the null hypothesis can be rejected. The range of difference in case two and three showed that although the results cannot be said to be statistically significant, there is better than a .5 probability that the results were not due to chance.

TABLE 2. Student's t-test expressing the range of eye muscle counts obtained for each of the three groups of mice infected with *Trichinella spiralis*

Case	Group	t value	Probability	Increase of infec. in %	Mean ratio dphm. wt. to collective eye muscle wt.
1	A-B	1.02	$.15 > p < .2$	127%	1:14.3
2	B-C	.886	$.35 > p < .5$	17%	1:16.7
3	A-C	3.16	$.005 > p < .01$	166%	1:16.6

Table 2 shows the changes in infection as the number of Trichinella larvae introduced is increased. Although there was no proportional increase, the number of Trichinella

larvae found in the eye muscles did increase substantially as the dosage increased. It is possible that there is an upper limit as to the number of Trichinella larvae which will reproduce within the intestine, or that this limit may exist in the cardiovascular stages of larval development.

According to the data, the ratio of the diaphragm to the weight of the eye musculature can be expressed as approximately 1:15. This information must be viewed with considerable skepticism due to the small values from which they have been extracted.

The relationship between the number of trichinella larvae introduced into mice and the muscle encystation in the diaphragm and the eye musculature can be seen in figure 1.

The gram muscle tissue technique is widely used to determine the degree of infestation. The diaphragm and the eye musculature were weighed to the nearest tenth of a milligram. In figure 2, the relationship between the number of larvae per gram of muscle tissue for both the diaphragm and the eye musculature is shown.

The results vary somewhat from results from the total muscle counts shown in figure 1. Using the gram muscle technique, the mice given 300 larvae per dose decreased in mean larval counts in the diaphragm. The eye muscle data varied little using either the whole muscle or the gram muscle technique.

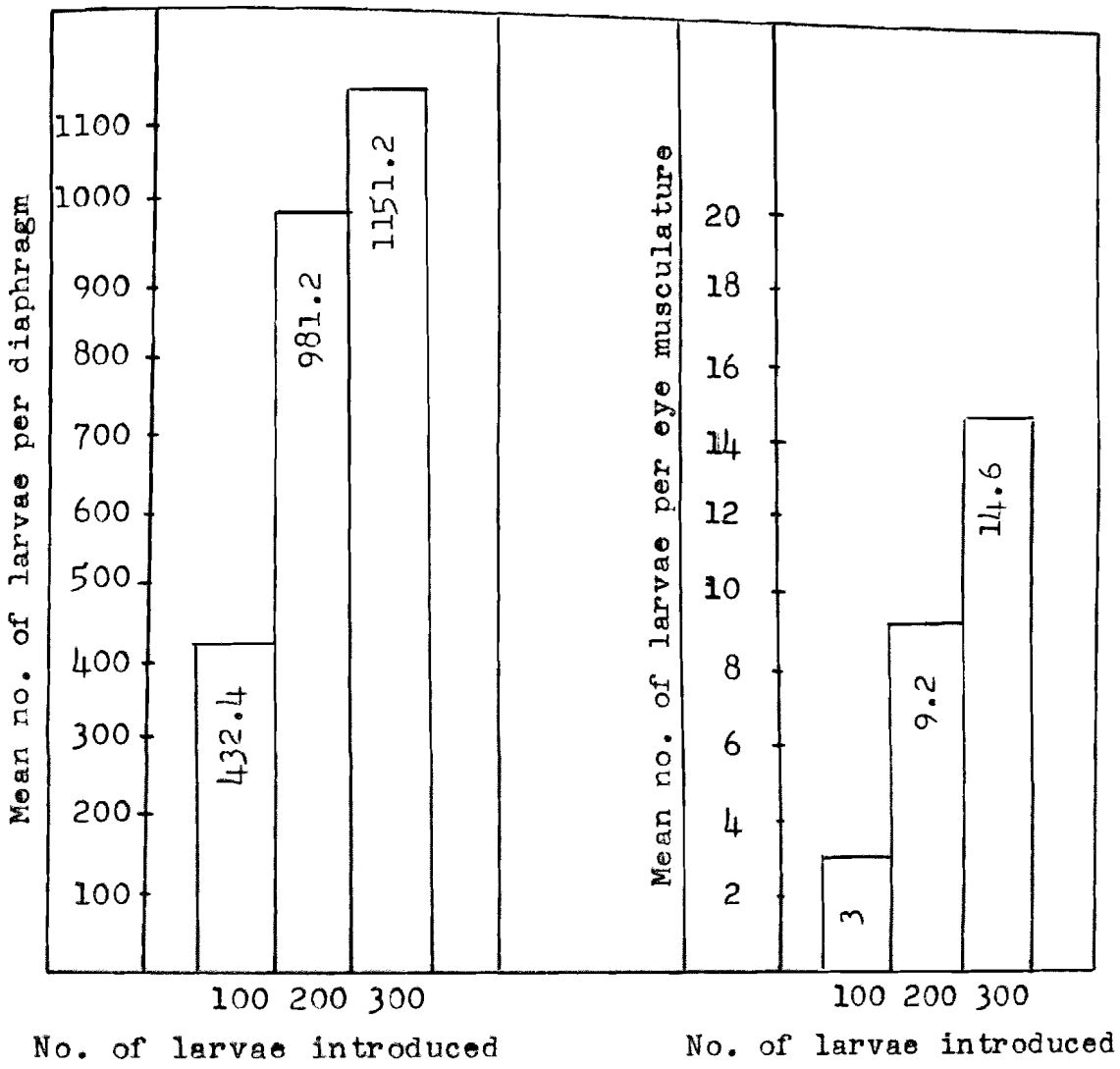

FIGURE 1. Average number of Trichinella spiralis larvae encysted in eyes and diaphragm

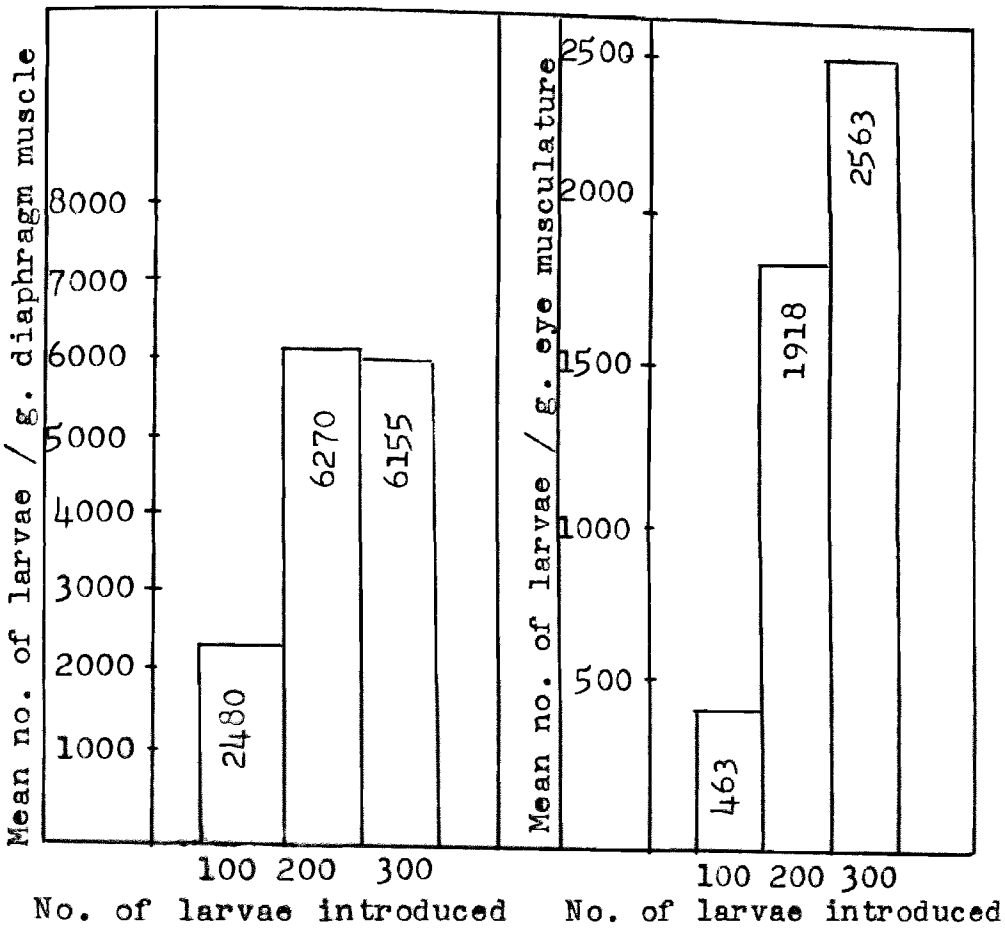

FIGURE 2. Mean number of Trichinella spiralis larvae per gram in the diaphragm and eye muscles

If the mean results are compared with the work done by Schoop et al. (1961), the present study finds significantly fewer larvae in the eye muscle of mice infected with 100 larvae. This present study shows a mean larval count of 3 as compared to 35 as found by Schoop et al. (1961). Even with massive doses of 300 larvae, only a mean of 14.6 were found. Although not mentioned, it must be assumed that Schoop et al. (1961), included everything within the eye orbital. In spite of this inclusion, the results found in this study seem to be in contradiction with previous investigations.

The data shows that 11 out of 15 cases, the mice with large diaphragm counts within the same group had a corresponding higher eye muscle count. This similarity was by no means proportional but because of the small size of the eye muscle, it is a valid relationship.

Trichinella larvae are transported to the skeletal muscles by the circulatory system. The distribution of these larvae to the rectus eye muscles varied greatly. Figures 3 and 4 indicate the total number of larvae in each of the rectus eye muscles.

The superior rectus is the largest of the rectus muscles (Alpern, 1962) and had the greatest number of larvae in comparison to the other three. The inferior, medial, and lateral rectus vary slightly in size and larval encystment and all are exceeded by the superior rectus.

The number of larvae introduced into the host animal was compared to the degree of infection found in each of the extra ocular eye muscles. The results found by determining the number of larvae per gram of muscle varied from the results found by using the total musculature. In all cases, the eye muscles of mice infected with 100 larvae have the least number of encysted larvae. The lateral and medial rectus were more highly parasitized in the mice infected with 100 larvae than those infected with 300 larvae.

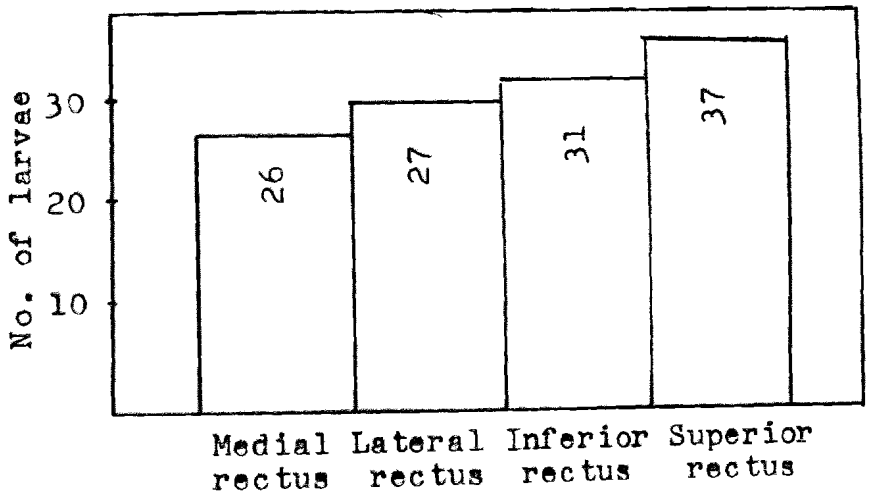

FIGURE 3. Total number of trichinella in the individual eye muscles (medial, lateral, inferior, superior rectus)

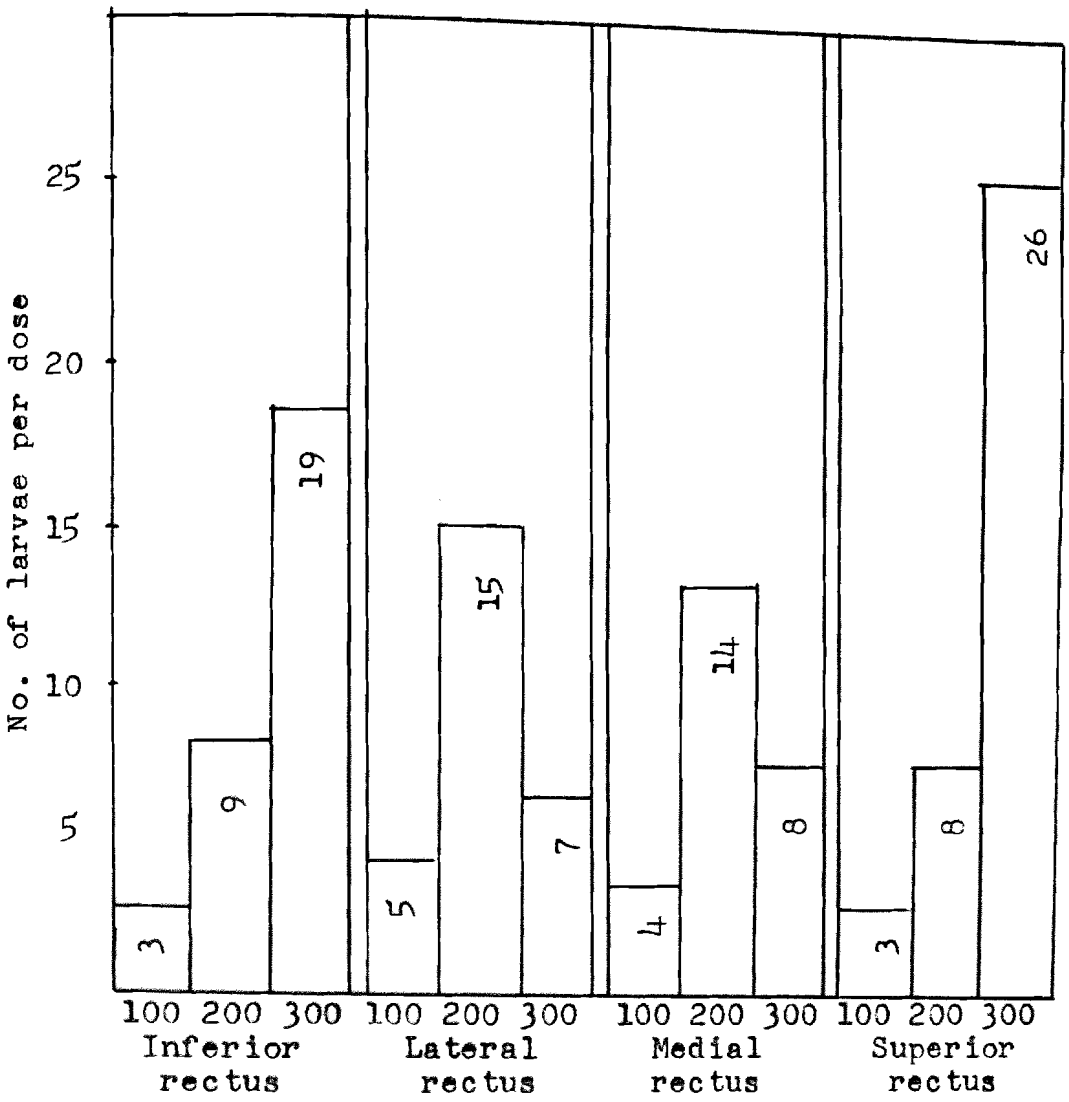

FIGURE 4. Total number of encysted larvae in the individual eye muscles of mice infected with varying numbers of Trichinella spiralis larvae

As the mice were dissected, a careful note was kept as to the condition and distribution of larvae within the individual rectus eye muscles. Both encapsulated and non-encapsulated larvae were found. Of 121 larvae found in the eye muscles, 8 were not encapsulated. Non-encapsulated larvae occurred in each of the four rectus muscles. According to Olson et al. (1964), the larvae encapsulate primarily in the belly of the muscle. Table 3 shows the number of larvae found in the belly of the muscle and at the end near the ligament.

TABLE 3. Distribution of trichinella larvae within individual eye muscles

Inferior rectus		Lateral rectus		Medial rectus		Superior rectus		t value	Probability
Belly	Lig.	Belly	Lig.	Belly	Lig.	Belly	Lig.		
20	11	20	7	10	16	29	8	2.28	.05>p<.1

The number of larvae found in the belly of the muscle exceeded that found near the ligament. The ratio of belly versus ligament larvae was 2:1. The medial rectus was an exception. There were more larvae found near the ligament than in the belly of muscle. Because the eye muscles are so small, the difference between the belly and the ligament sometimes varies. Using the t-test of significance, it was

found that the range of difference between those larvae found in the belly and those found in the ligament was not significant. The distribution within the muscle would appear to be random. Of the larvae, 79 were found in the center belly portion with 42 near the ligament.

CONCLUSION

The larvae of Trichinella spiralis migrate to the eye muscles of the laboratory mouse. It is generally agreed that virtually all skeletal muscle is infected by the migrating larvae as they pass through the systemic circulation. Oliver (1961), lists 45 muscles and muscle groups infected in the laboratory rat. The larvae migrate to each of the four rectus eye muscles. Although there were many cases where the individual eye muscles were not parasitized, larvae were found in the eye musculature of all the mice used in this study. The largest of these muscles, the superior rectus, was most highly parasitized. It was concluded that the eye musculature in the laboratory white mouse is a reliable indicator of trichinosis infection. This would agree with the study of Oliver (1961), and is contrary to the work done by Schoop et al. (1961). In the latter study, some mice had eyes that were not parasitized. Because the degree of infestation was much lower in the present study, the question posed is where do all of the larvae indicated by

Oliver (1961), and Schoop et al. (1961), reside? It seems that two remaining extra-ocular eye muscles would not account for all the remaining larvae. Some larvae counted by these studies might have been found in the connective tissue or the eye itself.

The correlation between the weight of the mouse and the corresponding weights of the diaphragm and eye musculature was not determined. A future study might determine this correlation by modifying the procedure used in this study.

The intensity of infection between eye muscles and the diaphragm was determined. In the present study, the ratio of diaphragm larvae count to eye muscle larvae count was approximately 4:1. This is slightly less than the 5:1 ratio found by Oliver (1961), using rats. The diaphragm has been used for many years in trichinoscopic inspection of pork. It is concluded that because of the degree of infectivity (Oliver, 1961; Schoop et al. 1961) and the reliability of determining Trichinella infection using eye muscles, this method could be used to inspect pork. This would be of advantage as the body cavity would not have to be opened as is the case when the diaphragm is used. Another possibility for the use of eye musculature is for postmortum human studies. The eye musculature could be used to determine the frequency of the disease in the human population.

There was very little difference in the results using either the whole muscle or the gram muscle technique.

The gram muscle technique could be used in the larger mammals. Blair et al. (1969), agrees with these findings. The greatest intensity of encysted larvae occurred in the belly of the muscle and if the gram muscle technique was to be used for trichinoscopic studies, the belly of the eye muscle should be used.

There is a definite correlation between the number of larvae encysted and the number of larvae found in the eye musculature. The question as to the correlation of muscle activity and degree of infestation was not determined. This type of information might be of value to kinesiologists as well as parasitologists.

Information is lacking to explain the actual distribution of larvae in the muscle system. Because each of the rectus eye muscles were parasitized within limits which exclude a predeliction to one or the other eye muscle, it is considered that the larvae are distributed randomly. The question still remains as to what is the attraction of the larvae to the striated muscle. It is recommended that further studies be done using the pig and human, or other species of animals so that the reliability of the eye muscle technique can be extended.

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